SEPARATION OF 2-(3-CHLOROPHENOXY) PROPIONIC ACID USING CAPILLARY ELECTROPHORESIS

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DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations, summaries and references each of which have been fully acknowledged.

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ABSTRACT

The separation of L,D-2-(3-chlorophenoxy)propionic acid mixture has been resolved by using capillary electrophoresis in capillary zone electrophoresis (CZE) mode. The wavelength detection was at 230 nm. The use of 50mM sodium dihydrogen phosphate buffer added with 10mM β-CD at pH 8.36 together with different applied voltage (10, 15, 20 kV) at a temperature of 30°C have been investigated. The results showed chiral separation of 2-(3-chlorophenoxy)propionic acid has been partly resolved by using 50mM sodium dihydrogen phosphate (pH 8.36) with 10mM β-CD at 30 °C at 10, 15, 20 kV. Unfortunately, further attempts to improve the resolution of the mixture could not be performed in this study due to limited amount of CD and non-availability of other potential chiral selectors. Beside that, persistent capillary electrophoresis technical problems were encountered during this study.

PEMISAHAN ASID 2–(3–CHLOROPHENOXY)PROPIONIC DENGAN MENGGUNAKAN KAPILARI ELEKTROFORESIS.

ABSTRAK

Pemisahan campuran asid L,D-2–(3–Chlorophenoxy)propionic telah dipisahkan dengan menggunakan kapilari elektroforesis dalam mod zon kapilari elektroforesis. Gelombang yang digunakan untuk mengesan adalah 230 nm. Kajian ini menggunakan 50 mM larutan penimbal natrium dihidrogen fosfat dengan 10mM β-CD pada pH 8.36 menggunakan aplikasi volatan yang berbeza (10, 15, 20 kV) pada suhu 30°C. Hasil keputusan menunjukkan bahawa pemisahan kiral 2-(3-chlorophenoxy)propionic asid sebahagiannya boleh diselesaikan dengan menggunakan 50mM natrium dihidrogen fosfat (pH 8.36) dengan 10mM β-CD at 30°C at 10, 15, 20 kV. Tetapi malangnya, percubaan yang seterusnya untuk memperbaiki resolusi terhadap campuran tersebut juga tidak dapat dijalankan dalam kajian ini kerana kuantiti CD yang terhad dan ketiadaan potensi pemilih kiral. Selain itu, masalah teknikal kapilari elektroforesis juga sering berlaku sepanjang menjalankan kajian ini.

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LIST OF SYMBOLS, ABBREVIATIONS AND UNITS

CE	Capillary Electrophoresis
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
SFC	Supercritical Fluid Chromatography
CDs	Cyclodextrins
S	Sulfur
P	Phosphorus
N	Nitrogen
Si	Silicone
PPA	Phenoxypropionic Acids
MSDS	Material Safety Data Sheet
CPA	Chlorophenoxy Acid
LD50	Lethal dosage 50
Mm	Micrometer
i.d.	Internal diameter
UV	Ultraviolet
kV	Kilovolt
CZE	Capillary Zone Electrophoresis
CITP	Capillary Isotachophoresis
CGE	Capillary Gel Electrophoresis
CIEF	Capillary Isoelectric Focusing
MEKC	Micellar Electrokinetic Chromatography
MECC	Micellar Electrokinetic Capillary Chromatography
CDEKC	Cyclodextrin Electrokinetic Chromatography
IXEKC	Ion Exchage Electrokinetic Chromatography
MEEKC	Microemulsion EKC
DNA	Deoxynucleic acid
V_{eo}	electroosmotic velocity
V_{ep}	electrophoretic velocity

α-CD	Alpha cyclodextrin
β-CD	Beta cyclodextrin
γ-CD	Gama cyclodextrin
°C	degree Celcius
CE-UV- visible	capillary electrophoresis-ultraviolet-visible
CE-ESI-MS	capillary electrophoresis-electron spray ionization
LC	liquid chromatography
VCD	vibrational circular dichroism
Nm	Nanometer
Pd	Palladium
C	Carbon
Raney-Ni	Raney-Nickel
mM	Millimeter
MLC	Micellar Liquid Chromatography
QSERs	Quantitative Structure-Enantioselectivity
Min	Minute
IL	Ionic Liquids
CE-MS	Capillary electrophoresis-mass spectrometry
CE-ESI-MS	capillary electrophoresis-eloectron spray ionization-mass spectrometry
CD-CZE-ESI-MS	capillary electrophoresis-capillary zone electrophoresis-eloectron spray ionization-mass spectrometry
NG	n-nonyl-β-$_D$-glucopyranoside
OG	n-octyl-β-$_D$-glucopyranoside
λ	Wavelength
MCPA	Mecoprop
CPPA	chlorophenoxy propionic acid
DL	Detection Limits
v/v	volume / volume
s	Second
Na OH	sodium hydroxide
μA	microAmpere
BGE	Background electrolyte

CHAPTER 1

INTRODUCTION

1.1 Overview

Enantiomeric separation is one of the most challenging tasks for any analytical technique including CE. There is a vast increased amount of publications related to this topic since the first report in 1985 showing the great possibilities of CE for the separation of chiral compounds (Hernandez-Borges *et al.*, 2005). Recently, there is a review on 2007 by Chankvetadze on the past, present and future of performing enantioseparations by using this technique. This article discussed the historical overview, fundamental aspects of enantioseparations using CE and the applications of using CE.

Traditionally, the techniques most frequently used for chiral separations have been GC, HPLC or supercritical fluid chromatography (SFC). In the last decade, CE has shown to be a powerful and versatile technique for a wide variety of chiral separation (Hernandez-Borges *et al.*, 2005) for chiral separation if compared to more conventional chromatographic techniques. This is due to its interesting characteristics

as high separation efficiency, speed of analysis, versatility, and low consumption of chiral selectors (Garcia-Ruiz *et al.*, 2005). Besides that, CE also allowing the incorporation of various chiral selectors at different concentrations while chiral selectors are usually fixed onto stationary phase in HPLC. Hence, the concentrations of the chiral selectors cannot be varied. An enantiomeric separation in CE is not based on an electrophoretic mechanism because the electrophoretic mobilities of the enantiomers of a chiral compound are equal and non selective (Garcia-Luiz *et al.*, 2005). In fact, the enantioselective recognition of the enantiomers of a chiral compound is due to their different interaction with a chiral selector (Garcia-Luiz *et al.*, 2005). Thus, it was caused by a chromatographic mechanism (Garcia-Luiz *et al.*, 2005).

Development of methods for chiral analysis by capillary electrophoresis methods has drawn a lot of attention in the previous decade (Lucangioli *et al.*, 2005). Capillary electrophoresis methods in its different modes have came out being an effective tool for the separation of enantiomers (Lucangioli *et al.*, 2005). This is due to its high efficiency and resolution in little time of analysis is the applicable features (Lucangioli *et al.*, 2005). For an example, methods with pseudostationary phases based on micelles and microemulsions or even on soluble additives like cyclodextrins which used to resolve chiral compounds have been reported (Lucangioli *et al.*, 2005).

In CE, separation method based on the difference rate at which analyte of ion migrate under influence of an electric field. CE carried out in buffer - filled capillary tube (Skoog *et al.*, 1998). The sample introduced into one end tubing and potential in certain voltage range applied. Electrophoresis is a separation method based on the

differential rate of migration of charged species in a buffer solution across (Skoog *et al.*, 1998).

The most general approach for direct enantiomeric separation in CE involves the addition of cyclodextrins (CDs) to the running buffer (Matthijs *et al.*, 2004). The purpose of these CDs is to improve the recognition properties by increasing the asymmetry and allowing for more specific interactions between the host and the guest such as hydrogen bonding and electrostatic interactions (Lin *et al.*, 2004). The use of cyclodextrins has shown an enormous potential in resolving enantiomeric separation of enantiomers with planar or axial asymmetry or those with heteroatoms (S, P, N and Si) in chiral centers (Juvancz & Szejtli, 2002). Since the CD was so useful, the applications of CDs were expanded with the introduction of CDs derivatives such as hydroxyalkyl-substituted CDs, sulfo- substituted CDs, carboxyl-substituted CDs, amino- substituted CDs and phosphate- substituted CDs. This can be proven as there are many research that use CD derivatives on the enantiomeric separation of compounds of environmental interest by CE in different works of previous research. CD derivatives have been proved for better solubility in various buffers and broader chiral recognition spectra than native CDs (Koppenhoefer, 2000).

Nowadays, pesticides constitute a class of important pollutants that are widely spread over the environment. About 25% of agrochemicals that exist contain chiral centers and are produced and used as racemic mixture. In some cases, there is only one of the isomers is active as pesticide while the others may have less activity or even toxic effects against nontarget organisms (Schmitt *et al.*, 1997). For an example, phenoxy acid herbicides (R)-isomers show much higher herbicide activity and

different metabolism than their (S)-isomers. In fact, only (+)-isomers of dichlorpop, mecoprop and diclofop-methyl are active as herbicides (Hernandez-Borges *et al.*, 2005). The use of racemates will contribute to useless environmental loading. Therefore, the interest in analytical techniques for the separation of environmental chiral compounds is increasing due to the importance of understanding enantiomeric discrimination in environment (Schmitt *et al.*, 1997).

1.2 Objectives

The aim of this study was,

1) To investigate the separation of 2-(3-chlorophenoxy)propionic acid at different concentration of cyclodextrins, concentration of background electrolyte buffer, pH and applied voltage.

1.3 Scope of study

In this study, enantiomeric separation of 2-(3-chlorophenoxy)propionic acid will be separated by using capillary electrophoresis (CE) Beckman Coultier. This enantiomeric standard compound will be analyzed in the CE instrument by using different experimental conditions such as cyclodextrins (CDs) concentration, buffer concentration, pH and voltage in order to optimize the enantiomeric separation. It is very important to separate enantiomers because they have different biological activities and this will result in different biological effects. For an example, the enantiomers are degraded at different rates when it is used as herbicides.

CHAPTER 2

LITERATURE REVIEW

2.1 Herbicides

Pesticides are extensively used in agriculture and herbicides represent the most prominent class throughout the world. Herbicides are phtotoxic chemicals have been frequently used in up-to-date agrochemical practice to increase the yield of various crops by inhibiting the growth or destroying different weeds. They have a range of degree of specificity. For an example, paraquat kills all green plants while phenoxy compounds are specific for certain category of plants.

2.1.1 Phenoxy acid Herbicides

Phenoxypropionic acids (PPA) are a chemical class of herbicides and are the oldest group of synthetic herbicides. They are important agrochemicals that show auxin-like activity which used in control of weeds in cereal crops. The molecular basis of the mode of action of PPA is not entirely understood. It was assumed that they are uncouplers of the oxidative phosphorylation system and modify the structure of

thylakoidal membranes (Cserhati & Forgacs, 1998). The PPA can move in agricultural ecosystems because of their solubility in water. This has caused the pollution of surface and ground waters. They are relatively less persistent in soil and water and can accumulate in river and lake sediments. PPA show moderate toxicity but some chlorinated metabolites can be toxic to human and aquatic organisms. It was reported that they can cause soft tissue carcinoma in man and show embryotoxicity in animals (Cserhati & Forgacs, 1998).

All the derivatives of PPA herbicides contain a phenolic ring with a chloro substituent. The oxygen of the phenolic ring is linked to an asymmetric carbon atom, leading to two optical isomers. It is believed that the R form of PPAs posseses the major herbicidal activity while the S form is herbicidally inactive.

2.1.2 2-(3-Chlorophenoxy)Propionic Acid

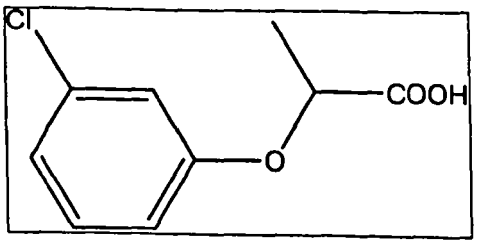

Figure 2.1 Structure of 2-(3-Chlorophenoxy) Propionic Acid

(Sources from Wolbach *et al.*, 2001)

MSDS name is DL-2-(m-Chlorophenoxy)propionic acid, 98%. The other name is Cloprop, 2-(m-Chlorophenoxy)propionic acid, 2-(3-Chlorophenoxy)propanoic acid, 3-CPA, 021202 (US EPA PC Code) 53404-22-1 (CAS Number). It is in light brown

powder and the freezing/melting point is 113.00 - 115.00 °C. The molecular formula is $C_9H_9ClO_3$ and its molecular weight is 200.62. It is stable under normal temperatures and pressures. The hazardous decomposition products are hydrogen chloride, carbon monoxide, carbon monoxide, carbon dioxide. The LD50 of rat (oral) is >750 mg/kg and the LD50 of rabbit (skin) is >2 gm/kg.

2.2 Capillary Electrophoresis (CE)

Electrophoresis is one of the most extensively used separation technique and still a rich field of innovative research although the theorectical principles are known for almost 100 years by Kohlrausch's work about the derivation of basic equations for ionic migration in an electrolyte solution in 1987 (Kuhn & Hoffstetter-Kuhn, 1993). Technically, electrophoresis is defined as a process for separating charged molecules based on their movement through a fluid under the influence of an applied electric field (Kuhn & Hoffstetter-Kuhn, 1993).

Capillary electrophoresis (CE) is a family of related techniques that employ narrow bore (20-200 μm i.d.) capillaries to perform high efficiency separation of a variety of analytes of different sizes, charge and whether the compound is simple molecules, organic and inorganic ions, peptides, proteins, carbohydrates and nucleic acid. These separations are facilitated by the use of high voltages. This may generate electroosmotic and electrophoretic flow of buffer solutions and ionic species respectively within the capillary. The separation technique is the most recently introduced electrophoretic technique but totally different from conventional electrophoresis. This is because the use of a supporting medium is not necessary and

analysis can be carried out in free aqueous solution (Camilleri, 1998). In some cases, the same capillary can be used for a variety of structural compounds by performing the consecutive electrophoresis. But, the electrophoresis must be run under different buffer conditions, varying buffer composition and/or adjusting pH.

2.2.1 Instrumentation

The experimental setup for CE is simple. From the figure 2.4, a capillary is required for the separation, a controllable high voltage power supply is required to drive the separation, two electrode assemblies, two buffer reservoirs, an ultraviolet (UV) detector is required to determine the presence and amount of analyte and last but not least is a safety interlocks equipped enclosure which is used to protect the operator from high voltage.

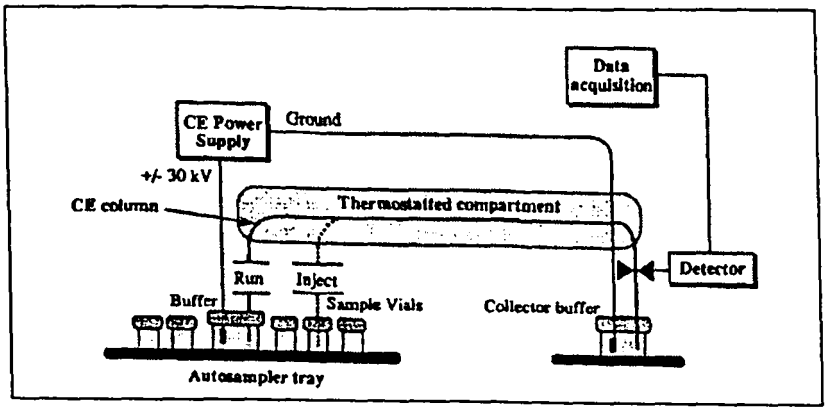

Figure 2.2 Schematic representation of a basic capillary electrophoresis instrument.

Source from (Thibault & Dovichi,1998)

Normally the high voltage direct current power supply delivers up to 30 kV. It is connected to two platinum electrodes extending into buffer solution while the ends of the capillary tube (20 to 200 μm i.d.) are dipped in the two buffer containers and the optical viewing window is aligned with the detector. After filling the capillary with buffer, the sample can be introduced by dipping the end of the capillary into the sample. Then the immersed capillary is elevating a foot or so above the detector-side buffer reservoir. Separation will occur once the immersed capillary is inside the running buffer with the applied voltage to separate the sample. Signals are electronically collected, stored and visually displayed on a recorder or printer (Camilleri, 1998).

2.3 Modes of Capillary Electrophoresis

Nowadays, CE has become a mature analytical technique providing practical and innovative solutions to challenging separation problems. This is due to the versatility and range of applications of CE is deriving from its unique characteristics and advantages compared to other complementary analytical techniques (Camilleri, 1998). CE comprises a family of techniques that have a great number of systems involving either differential or equilibrium gradient methods (Kuhn & Hoffstetter-Kuhn., 1993). Currently, there are five modes of CE is capillary zone electrophoresis (CZE), capillary isotachophoresis (CITP), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF) and micellar electrokinetic chromatography (MEKC).

2.3.1 Micellar Electrokinetic Chromatography (MEKC)

This capillary electrophoresis technique was introduced by Terabe *et al.*, (1984) and they referred this technique as 'Electrokinetic separation'. In this paper, sodium dodecyl sulfate was employed as a micelle-forming reagent and it was the first one that published in 1984. Later, in their second paper (Terabe *et al.*, 1985) on the technique, they used the term 'electrokinetic chromatography' because they felt that it is a type of chromatography similar to liquid-liquid partition chromatography. This mode of capillary electrophoresis is then referred to as 'micellar electrokinetic chromatography' or MEKC. This technique was developed to help resolve neutral solutes that were not separable by CZE and also allows the resolution of charged solutes (Demarest *et al.*, 1992).

In the literature, MEKC is also referred to as micellar electrokinetic capillary chromatography (MECC) since the separations are most often performed in a capillary tube. Other modes of EKC are cyclodextrin EKC (CDEKC), ion-exchange EKC (IXEKC) and microemulsion EKC (MEEKC). Cyclodextrin derivatives, polymer ions and microemulsions are used in CDEKC, IXEKC and MEEKC respectively instead of the micelles used in MEKC. All EKC techniques are based on the same separation principle that is the differential partitioning of an analyte between two-phase systems such as mobile or aqueous phase and a stationary phase (Beckman, 1994)

The same instrument that is used for CZE is also used for MEKC. MEKC is different in that it uses an ionic micellar solution instead of the simple buffer salt solution used in CZE. The micellar solution generally has a higher conductivity and

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